REMARKS

Upon entry of this amendment, claims 1-17 are pending. Claims 1 and 4 have been amended. Claims 7-17 have been added. No claims have been canceled.

Support for the amendment to claim 1 appears at least at claim 1, ¶0049, and ¶0085. Support for the amendment to claim 4 appears at least at ¶0049 and ¶0085.

Support for new claim 7 appears at least at ¶0049. Support for new claims 8 and 9 appears at least at ¶0085 and ¶0086. Support for new claim 10 appears at least at ¶0047. Support for new claims 11 and 12 appears at least at ¶0048. Support for new claims 13 and 14 appears at least at ¶0035. Support for new claim 15 appears at least at ¶0051. Support for new claim 16 appears at least claim 1, ¶0035, ¶0047, ¶0085, ¶0086, and ¶0048. Support for new claim 17 appears at least at claim 2.

No new matter has been added by way of this response.

Claim Rejections under 35 U.S.C. § 112

Applicants respectfully traverse and, for the following reasons, request reconsideration and withdrawal of the rejection of claims 1-3 under 35 U.S.C. § 112 as being indefinite. The Office asserts that a "base sequence and a target base sequence are single base nucleotides" and that, as such, "they may be considered point mutations". The Office further maintains it is "not clear what is meant by having a mutation site in the context of said single base".

Definiteness of claim language must be analyzed, not in a vacuum, but in light of the content of the particular application disclosure, the teachings of the prior art, and the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. MPEP § 2173.02. A claim may not be rejected solely because of the type of language used to define the subject matter for which patent protection is sought. MPEP § 2173.01. The Office's focus must be on whether the claim meets the threshold requirements of clarity and precision, not whether more suitable language or modes of expression are available. MPEP § 2173.02.

Applicants respectfully assert that the Office is misinterpreting "target base sequence". The specification, in ¶0035, recites that:

> The target base sequence to be detected is appropriately selected depending on the type of an amplification method so that the target base sequence having a mutation site can be specifically amplified. In general, the mutation site contained in the target base sequence to be detected is known as a site of gene mutation

> or gene polymorphism. The mutation of the site may be a point mutation, an insertion mutation or a deletion mutation.

From the above passage, it is clear that a target base sequence, as that term is used in the present application, is not a single base nucleotide (as asserted by the Office), but rather a polynucleotide sequence (*i.e.*, a sequence of bases) containing a mutation site. The above passage also makes clear that the scope of the claim is not limited to a "point mutation" as suggested by the Office, but also encompasses other types of mutations, such as an insertion mutation or a deletion mutation. Furthermore, it is generally understood in the art that a "base sequence" is synonymous with terms such as a "sequence of bases" or a "polynucleotide sequence".

In summary, given the understanding in the art, the specification disclosure, and the claim language, one skilled in the art would clearly understand the boundaries of the subject matter for which protection is sought. As such, claims 1-3 are not indefinite.

Claim Rejections under 35 U.S.C. §103(a) over Lay in view of Klepp

Applicants respectfully traverse and, for the following reasons, request reconsideration and withdrawal of the rejection of claims 1, 3, 4, and 6 under 35 U.S.C. §103(a) as being unpatentable over Lay et al. (1997) Clin Chem 43(12), 2262-2267 ("Lay") in view of Klepp et al. (2000) Biochemica 2, 14-16 ("Klepp").

To establish a *prima facie* case of obviousness, the prior art reference (or references when combined) must teach or suggest all the claim limitations; there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; and there must be a reasonable expectation of success. MPEP § 2143.

The present invention is directed to chromatographic detection of polynucleotide sequences having mutation sites via differentially labeled primers and hybridization probes. The hybridization probes are generally shorter than those conventionally used in the art for allele specific oligonucleotide hybridization (*see* ¶0049). The shorter length hybridization probe provides, for example, (i) difference in Tm due to mismatch of a single base larger than that of a longer probe thus increasing specificity, (ii) lower hybridization temperatures allowing room temperature subsequent procedures, and/or (iii) reduced Tm values such that the probe does not hybridize during the DNA amplification reaction (*see* ¶0053).

Claim 1 requires, inter alia, "amplifying DNA containing a target base sequence to be detected having a mutation site using DNA polymerase; hybridizing the amplified DNA to a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected; and detecting a hybrid formed by the hybridization, wherein at least one of primers to be used in the DNA amplification is labeled with a first labeling agent so that the amplified DNA will be labeled with the first labeling agent, the hybridization probe is labeled with a second labeling agent and contained in a reaction solution for effecting the DNA amplification, the base sequence of the hybridization probe is designed not to inhibit the DNA amplification, and the hybrid is detected by affinity chromatography with the use of the first and second labeling agents."

Lay does not teach or suggest all elements of claim 1

Lay is directed to a *real time* method for fluorescence genotyping in which resonance energy transfer between two adjacent hybridization probes is monitored as the temperature changes during PCR, where a sharp decrease in fluorescence is observed around the melting temperature of the probes. According to the method of Lay, if one fluorophore is on a primer and the other on an internal hybridization probe, the resonance energy transfer depends only on hybridization of the single internal probe. This allows the *melting characteristics* of the internal probe to be monitored *during amplification*. Sequence alterations in the target at the probe site can be detected because the stability of the duplex and hence the melting temperature of the probe decrease when mismatches are present.

Lay does not teach or suggest all the features of claim 1. For example, Lay does not teach or suggest detecting a formed hybrid by affinity chromatography with the use of the first and second labeling agents, as required by claim 1. As another example, Lay does not teach or suggest hybridizing the amplified DNA to a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected, as required by claim 1.

Klepp does not teach or suggest all elements of claim 1

Klepp is directed to a multi-step method to detect the success of a PCR amplification using a digoxigenic- or biotin-labeled PCR product in conjunction with chromatographic test strip detection. Klepp teaches amplification of a DNA molecule with a labeled primer followed by a

separate hybridization reaction in which a labeled probe is added to an aliquot of the PCR reaction.

Klepp does not teach or suggest all the features of claim 1. For example, Klepp does not teach or suggest hybridizing the amplified DNA to a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected, as required by claim 1. In fact, Klepp teaches away (*see* MPEP § 2141.02(VI)) from such feature by requiring that the "size of the labeled oligonucleotide [hybridization probe] *must* range between 17 - 40 bases" (Klepp, p. 14, col. 2, ln. 7-8; emphasis added).

No motivation or suggestion to combine references

There is no suggestion or motivation to modify the methods of Lay or Klepp so as to reach the requirements of claim 1. Neither of the cited references recognize or suggest the use of shorter labeled hybridization probes as described in the present invention. And Klepp teaches away from the use of short probes with the requirement that probes of 17 - 40 bases must be used in the chromatographic methods described therein (*see* MPEP § 2141.02(VI)). Thus, neither cited reference provides a suggestion or motivation to use shorter labeled hybridization probes; nor does either reference provide a reasonable expectation of success that such shorter hybridization probes would work in the described methods, or in any method for allele specific oligonucleotide hybridization.

Furthermore, modifying the differentially labeled primer and probe method of Lay with the chromatographic detection of Klepp would make the method of Lay unsuitable for its intended purpose. Under MPEP § 2143.01(V), if a proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. Lay is directed to a "real-time" genotyping method in which differential fluorescence labeling of primer and probe provides for genotyping *during amplification* (i.e., real-time) (see e.g., title, abstract, discussion at p. 2265, col. 2, ln. 1-7, 13-25). Substitution of the test strip affinity chromatographic detection of Klepp into the real-time fluorescence genotyping method of Lay would remove the real-time aspect of the Lay. Because the proposed modification would render the method of Lay unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the modification proposed by the Office.

The above argument applies equally to claim 1 and claims dependent thereon, such as claim 3. The above argument applies equally to claim 4 and claims dependent thereon, such as claim 6, to the extent such claims require a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected, a primer labeled with a first labeling agent, a hybridization probe labeled with a second labeling agent, and detection by affinity chromatography with the use of the first and second labeling agents.

Claim Rejections under 35 U.S.C. §103(a) over Lay in view of Klepp and Gunnenberg

Applicants respectfully traverse and, for the following reasons, request reconsideration and withdrawal of the rejection of claims 2 and 5 under 35 U.S.C. § 103(a) as being unpatentable over Lay in view of Klepp and Gunneberg et al. (1993) Clin Chem 39(10) 2157-2162 ("Gunnenberg").

Claim 2 incorporates all features of claim 1 and further requires that "the mutation site is a point mutation, and the reaction solution for effecting the DNA amplification further contains an unlabeled oligonucleotide having a base sequence different in a single base at the position of the point mutation from the base sequence of the labeled hybridization probe, in an amount sufficient to enhance the specificity of hybridization of the amplified DNA to the hybridization probe."

Lay does not teach or suggest all elements of claim 2

Lay does not teach or suggest all requirements of claim 2. For example, Lay does not teach or suggest detecting a formed hybrid by affinity chromatography with the use of the first and second labeling agents, as required by claim 2. As another example, Lay does not teach or suggest hybridizing the amplified DNA to a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected, as required by claim 2. As a further example, Lay does not teach or suggest an unlabeled oligonucleotide, differing by one base from the labeled hybridization probe at the point mutation, contained in the amplification reaction mixture such that the specificity of hybridization is increased, as required by claim 2.

Klepp does not teach or suggest all elements of claim 2

Klepp does not teach or suggest all requirements of claim 2. For example, Klepp does not teach or suggest hybridizing the amplified DNA to a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected, as required by claim 2. In fact, Klepp teaches away (*see* MPEP § 2141.02(VI)) from such feature by requiring that the "size of the labeled oligonucleotide [hybridization probe] *must* range between 17 - 40 bases" (Klepp, p. 14, col. 2, ln. 7-8. emphasis added). As a further example, Klepp does not teach or suggest an unlabeled oligonucleotide, differing by one base from the labeled hybridization probe at the point mutation, contained in the amplification reaction mixture such that the specificity of hybridization is increased, as required by claim 2.

Gunneberg does not teach or suggest all elements of claim 2

Gunneberg is directed to a genotyping method with improved signal to noise ratio. Gunneberg describes the use of PCR amplification of the α-antitrypsin gene followed by membrane blotting sequential hybridization with a radiolabeled 21 mer hybridization probe and an unlabeled 21 mer competitive hybridization probe, washing of the membrane to remove unbound probes, and autoradiography used to detect the hybridized radiolabeled probe.

Gunneberg does not teach or suggest all requirements of claim 2. For example, Gunneberg does not teach or suggest hybridizing the amplified DNA to a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected, as required by claim 2. As another example, Gunneberg does not teach or suggest including a labeled hybridization probe in the DNA amplification reaction solution, as required by claim 2. As a further example, Gunneberg does not teach or suggest including a competitive unlabeled hybridization probe in the DNA amplification reaction solution, as required by claim 2. Not only does Gunneberg require separate amplification and hybridization reactions, Gunneberg further requires sequential hybridization of the unlabeled and labeled probes. In fact, Gunneberg arguably teaches away from joint hybridization with a labeled probe and an unlabeled competitive probe by citing problems with previous studies that attempted simultaneous hybridization (see Gunneberg, p. 2161, in ¶ bridging col. 1-2).

No motivation or suggestion to combine references

There is no suggestion or motivation to modify the methods of Lay, Klepp, or Gunneberg so as to reach the requirements of claim 1. None of the cited references recognize or suggest the use of shorter labeled hybridization probes or unlabeled competitive hybridization probes as described in the present invention. And Klepp teaches away from the use of short probes with the requirement that probes of 17 - 40 bases must be used in the chromatographic methods described therein (*see* MPEP § 2141.02(VI)). Thus, none of the cited reference provide a suggestion or motivation to use shorter labeled hybridization probes or unlabeled competitive hybridization probes; nor does either reference provide a reasonable expectation of success that such shorter probes would work in the described methods, or in any method for allele specific oligonucleotide hybridization.

Furthermore, Gunneberg teaches away from including a labeled probe and an unlabeled competitive probe in the amplification reaction mixture and conducting the hybridization reactions jointly, rather than sequentially (*see* MPEP § 2141.02(VI)). And as pointed out above, Klepp teaches away from the use of short probes with the requirement that probes of 17 - 40 bases must be used in the chromatographic methods described therein.

And modifying the differentially labeled primer and probe method of Lay with the sequential hybridization of labeled probe and unlabeled competitive probe of Gunneberg and the chromatographic detection of Klepp would make the method of Lay unsuitable for its intended purpose. Under MPEP § 2143.01(V), if a proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. Lay is directed to a “real-time” genotyping method in which differential fluorescence labeling of primer and probe provides for genotyping *during amplification* (i.e., real-time) (see e.g., title, abstract, discussion at p. 2265, col. 2, ln. 1-7, 13-25). Substitution of the sequential hybridization of labeled probe and unlabeled competitive probe of Gunneberg into the real-time fluorescence genotyping method of Lay would remove the real-time aspect of the Lay because, first, hybridization is not conducted in the amplification reaction mixture, and second the hybridization does not occur simultaneously, but rather sequentially. Similarly, substitution of the test strip affinity chromatographic detection of Klepp into the real-time fluorescence genotyping method of Lay would likewise remove the real-time aspect of the Lay. Because the proposed modification would render the method of Lay unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the modification proposed by the Office.

The above argument applies equally to claim 5 to the extent such claim requires a hybridization probe with length of 10 - 15 mer having a base sequence complementary to the target base sequence to be detected, a primer labeled with a first labeling agent, a hybridization probe labeled with a second labeling agent, and detection by affinity chromatography with the use of the first and second labeling agents.

Other Claims

Applicants believe new claims 7-17 to be patentable over the cited references.

Claim 7 requires the length of the hybridization probe to be 10 - 13 mer. Lay, Klepp, and Gunneberg do not teach or suggest such feature, either alone or in any known combination, nor is there a motivation to alter the method of these references so as to reach the requirements of claim 7.

Claim 8 requires the length of the hybridization probe to be 11 - 13 mer. Lay, Klepp, and Gunneberg do not teach or suggest such feature, either alone or in any known combination, nor is there a motivation to alter the method of these references so as to reach the requirements of claim 8.

Claim 9 requires the length of the hybridization probe to be a 12 mer or a 13 mer. Lay, Klepp, and Gunneberg do not teach or suggest such feature, either alone or in any known combination, nor is there a motivation to alter the method of these references so as to reach the requirements of claim 9.

Claim 10 requires the length of the hybridization probe is selected so that hybridization to the target base sequence does not substantially occur at a temperature at which the DNA polymerase is actively amplifying the target base sequence. Lay, Klepp, and Gunneberg do not teach or suggest such feature, either alone or in any known combination, nor is there a motivation to alter the method of these references so as to reach the requirements of claim 10.

Claim 11 requires the Tm of the hybridization probe to be 25 to 40°C lower than the Tm of the labeled primer, and claim 12 requires the Tm of the hybridization probe to be 30 to 35°C lower than the Tm of the labeled primer. Lay, Klepp, and Gunneberg do not teach or suggest such features, either alone or in any known combination, nor is there a motivation to alter the method of these references so as to reach the requirements of claims 11 or 12.

Claim 13 requires the mutation site to be an insertion mutation, while claim 14 requires the mutation site to be a deletion mutation. Lay, Klepp, and Gunneberg (each directed to

single-point mutation) do not teach or suggest such features, either alone or in any known combination, nor is there a motivation to alter the method of these references so as to reach the requirements of claims 13 or 14.

Claim 15 requires the hybridization probe to contain a sequence complimentary to the mutation site of the target base sequence at about a middle of the hybridization probe. Lay, Klepp, and Gunneberg do not teach or suggest such feature, either alone or in any known combination, nor is there a motivation to alter the method of these references so as to reach the requirements of claim 15.

Claim 16 is directed to a method of detecting a target base sequence. Claim 16 requires amplifying DNA containing a target base sequence to be detected having a mutation site using DNA polymerase; hybridizing the amplified DNA to a 12 mer or 13 mer hybridization probe having a base sequence complementary to the target base sequence to be detected; and detecting a hybrid formed by the hybridization, wherein the mutation site of the target base sequence is selected from the group consisting of a point mutation, an insertion mutation, and a deletion mutation; at least one primer to be used in the DNA amplification is labeled with a first labeling agent so that the amplified DNA will be labeled with the first labeling agent; the hybridization probe is labeled with a second labeling agent and contained in a reaction solution for effecting the DNA amplification; the base sequence of the hybridization probe is designed not to inhibit the DNA amplification; hybridization to the target base sequence does not substantially occur at a temperature at which the DNA polymerase is actively amplifying the target base sequence; the Tm of the hybridization probe is 30 to 35°C lower than the Tm of the at least one labeled primer; and the hybrid is detected by affinity chromatography with the use of the first and second labeling agents. Lay, Klepp, and Gunneberg do not teach or suggest all such features, either alone or in any known combination, nor is there a motivation to alter the method of these references so as to reach all requirements of claim 16.

Claim 17 incorporates all requirements of claim 16 and further requires the mutation site to be a point mutation, and the reaction solution for effecting the DNA amplification to further contain an unlabeled oligonucleotide having a base sequence different in a single base at the position of the point mutation from the base sequence of the labeled hybridization probe, in an amount sufficient to enhance the specificity of hybridization of the amplified DNA to the hybridization probe. Lay, Klepp, and Gunneberg do not teach or suggest all such features, either alone or in any known combination, nor is there a motivation to alter the method of these references so as to reach all requirements of claim 17.

CONCLUSION

Applicant respectfully requests withdrawal of the rejections and believes that the claims as presented represent allowable subject matter. If the Examiner desires, Applicant welcomes a telephone interview to expedite prosecution. Applicant believes there is a fee for a three month extension of time and has enclosed a Credit Card Payment Form for $1020 to cover this fee. However, the Commissioner is hereby authorized to deduct any deficiency or credit any overpayment to Deposit Account No. 19-3140.

Respectfully submitted,

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